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Microcracks as tooth structural element: revealed by X-ray tomography and machine learning

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ABSTRACT

Although teeth microcracks have long been considered more of an aesthetic problem, their exact role in tooth structure and impact on its functionality is still unknown. The possibility to use X-ray micro-computed tomography for three-dimensional (3D) non-destructive tooth visualization has shown unique morphological and structural features of microcracks that previously could not be detected with two-dimensional analysis techniques. We present 3D evaluation of four healthy (2 with and 2 without visible microcracks on the buccal surface) extracted human teeth, scanned with ZEISS Xradia 520 Versa and segmented with convolutional neural network to identify enamel, dentin, and cracks. An intricate star-shaped network of microcracks was found to cover most of the inner tooth with the main crack planes arranged radially in two almost perpendicular directions, disclosing the crack as a planar rather than a threaded structure. The interconnected network of microcracks detected inside a healthy tooth suggests that the cracks could be considered as one of the structural and possibly functional (i.e. redistribution of forces) elements of the tooth, relevant for its integrity and functionality.

Introduction

Microcracks (MCs), which can be clearly visible on the outer surface of the tooth, have long been considered more of an aesthetic problem for patients, but their exact role and impact on the tooth integrity and longevity is still unidentified (typical images of teeth with MCs presented in Supplementary Fig. S1). To what extent patients’ concerns about the adverse effects of cracks on their teeth are justified, e.g. whether it is safe to bond brackets on cracked teeth, or whether dentists should take extra precautions when treating teeth with MCs – are questions that can’t be reasonably answered yet1. In the light of current knowledge, mainly based on a lateral (two-dimensional, 2D) analysis of the tooth surface, these MCs usually should not cross the dentin-enamel junction (DEJ) and should have no loss or visible separation of tooth structure2.

Although MCs are generally thought to be confined to the enamel, they are being associated not only with the damaged appearance of the teeth, but also with a variety of undesirable and pathological consequences, such as compromised integrity of the enamel, effect on the sensitivity of the teeth, stain and plaque accumulation on the rough fractured surface, and an increased susceptibility to carious lesions3–6. To date, the available information on teeth MCs and their characteristics has been obtained using 2D analysis techniques such as stereomicroscopy7–10, scanning electron microscopy (SEM)1,3,11 or three-dimensional (3D) scanning methods (optical coherence tomography (OCT) and ultrasound)12–16. The evaluation and measurement of qualitative and quantitative parameters of cracks (their number, direction, location, length, and width) presented in previously published literature describe the morphology and behaviour of MCs only on the outer enamel surface1,3,7–9,17–19. Thus, it is still unknown whether these cracks are confined to the enamel or they can extend beyond the DEJ to the dentin or even the pulp.

To satisfy curiosity and to accurately assess the extent of possible damage (i.e. whether a MC crosses the DEJ and reaches the dentin or the pulp, what is its path throughout the tooth) to the underlying tooth structures in the
would reduce the need for manual evaluation of radiographic images. Several studies have been carried out so far on the depth parameter of cracks. However, the limitations of the techniques used in those studies (e.g. the limited penetration depth and scanning range of the device utilized for MCs analysis, sensitivity of the technique to surface curvature, the depth measurements carried out on a simulated human tooth, an indirect method of determining the depth of the crack, the need for crack infiltration with contrast material for depth assessment, or the physical measurement of the crack after cutting the tooth) have all been the reasons behind the search and development of a new approach enclosing 3D imaging technique that would enable a non-destructive examination of MCs with micrometer resolution.

Advances in digital dentistry are followed by increasing attempts to computerise certain routine clinical procedures, particularly the analysis of radiographs. Artificial intelligence models for tooth and alveolar bone segmentation from cone-beam computed tomography images, classification of cervical maturation degree and pubertal growth spurts from lateral cephalometric radiographs would reduce the need for manual evaluation of radiographic images and contribute to treatment efficiency. However, to accurately visualise the tooth structure, a higher resolution 3D imaging technique is needed than has been used so far.

The introduction of an X-ray micro-computed tomography (µCT) in dental studies has opened up new possibilities for enamel thickness and tooth measurements, caries research, characterization of enamel white spot lesions and cortical bone microdamage, analysis of root canal morphology and preparation, detection of various types of teeth fractures, and dental tissue engineering. This is an accurate 3D imaging technique that utilizes X-rays to see inside an object, not limited to slice-by-slice views. One of the most important advantages of the method is the ability to provide volumetric information about the microstructure in a non-destructive way (today’s most advanced laboratory-based µCTs can achieve resolutions up to 0.7 –1 µm (4 –10 µm resolution usually selected for biological samples) using geometrical magnification), and generally eliminating an extensive sample preparation step.

In our recently published study X-ray µCT has been validated as a method suitable for the 3D non-destructive visualization of enamel MCs with distinct precision and versatility. However, it is assumed that the potential of this method is much broader and that the proposed approach is fully expandable towards the more detailed teeth microstructure analysis.

The aim of this study is to reveal the role of MCs in the integrity and functionality of a healthy (undamaged) tooth (with or without visible MCs on the outer surface) using X-ray µCT in combination with convolutional neural network (CNN) assisted voxel classification and volume segmentation. Network of microcracks had a typical cross sectional width (a local normal to the plane of crack) from ~0.3 –30 µm, which spans two orders of magnitude.

Results

Four healthy undamaged human maxillary premolars (with and without visible enamel MCs on the outer surface) that had been extracted for orthodontic reasons were analysed using µCT together with CNN assisted segmentation. X-ray images of all the samples showed a dense tooth structure in which enamel, dentin, pulp, and cracks could be identified. The teeth appeared to be cracked, but without visible damage or separation of fragments. The network of cracks found in all the healthy teeth examined suggests that the cracks, along with the enamel, dentin, and pulp, could be considered a structural element of the tooth. The summary of study is presented graphically in Fig. 1 with detailed results demonstrated below.

Characterization of microcracks

X-ray µCT in combination with CNN assisted segmentation allowed to characterize all tooth’s MCs in three dimensions (Fig. 2, see Supplementary Movie 1). It was possible to clearly identify the MCs located on the buccal, palatal, and contact surfaces of the tooth and to determine in which volume of the tooth (e.g. enamel or dentin) they initiate and extend (Fig. 3, see Supplementary Movie 2). The morphological characteristics of the different tooth surfaces, such as degree of convexity, surface roughness, enamel layer width, did not interfere with the MCs assessment procedure. The fact that the MCs were visible on the outer surface or buried deep inside the tooth had also no effect on their evaluation.

Evaluation of microcracks arrangement

The applied scanning technique and developed segmentation approach allowed us to analyze the arrangement of MCs. Cracks that connect to each other have been identified and differentiated from those that are isolated. A single network of star-shaped cracks (longitudinally in relation to the main axis of the tooth) was found to cover most of the internal tooth structure (Fig. 4, see Supplementary Movie 3). This continuous connective formation occupies...
Structural features of microcracks

The 3D visualization allowed the structural properties of MCs to be assessed. In the microcrack network, it was possible to distinguish the main planes of the crack in two almost perpendicular directions, thus revealing the crack as a planar (interconnected manifolds) rather than a threaded structure (Fig. 4).

All four teeth in the sample were characterised and evaluated using the same method (Fig. 5, Fig. 6, and Supplementary Movie 4).

Discussion

These findings demonstrate that approach for the evaluation of healthy human teeth (scanning with X-ray μCT and CNN assisted segmentation) is capable of characterizing tooth’s MCs regardless in which surface of the tooth they are and in which layer of the tooth they initiate and extend. Other studies have estimated crack parameters (measured by SEM, laser ultrasonic system, ultrasound, using indocyanine green near-infrared fluorescence and conventional near-infrared illumination): a) length range 0.24 – 10.15 mm; b) width range 0.25 – 35.04 µm; c) depth range 0.10 ± 0.01 mm of the craze lines; d) 0.8 – 1.0 mm in the cracked tooth; e) ~1.2 mm depth of the crack in simulated dentin; f) 0.658 – 0.717 mm crack depth calculated from the crack shadow, and g) 0.708 mm depth value physically measured by cutting the tooth.

The proposed method being insensitive to the curved surfaces of the study specimens overcomes the shortcomings of previous crack analysis techniques. This is particularly important when the subject is a tooth having four surfaces of different convexity, of which the buccal surface is the most commonly examined and also the most convex. Compared to the 3D scanning methods used so far (OCT, ultrasound), X-ray μCT allows the assessment of MCs at various distances from the outer enamel surface (enamel thickness ≈ 0.5 mm in the cervical region, up to ≈ 2.5 mm near the cusp for the molar teeth) or even in deeper layers of the tooth, e.g. the dentin, and is not affected by the different densities of these materials (density of enamel, 2.61 ± 0.04 g/cm³ – 2.77 ± 0.04 g/cm³, and dentin 1.79 ± 0.02 g/cm³ – 2.12 ± 0.03 g/cm³).

The aim of the study was to reveal the role of MCs in the integrity of an intact tooth (i.e. healthy, undamaged tooth with no previous orthodontic, endodontic or restorative treatment) by 3D examining the arrangement and structural features of cracks (i.e. whether they tend to be isolated or connected to each other to form larger clusters). A single network of star-shaped cracks was identified to cover most of the internal tooth structure. The tendency of MCs to interconnect as they extend deeply from the outer enamel (the enamel closest to the tooth’s surface where...
Figure 2. X-ray µCT data-cube projected density maps (bluish higher / brownish lower) along three major axes indicate convolutional neural network segmented voxels belonging to: a1–a3 the tooth, b1–b3 enamel, c1–c3 dentin, and d1–d3 cracks (colour intensity scaled as square root for enhancement).
Figure 3. Views of four surfaces (buccal, contact right, palatal, and contact left) of the tooth. a1–a4 Enamel surface and cracks (red), b1–b4 enamel removed, dentin surface is visible with cracks within the enamel volume (red), c1–c4 cracks on the dentin surface, and d1–d4 cracks within the dentin volume shaded as distance along line of sight (lighter red indicates closer / darker – further from the viewer).
the rods extend in a nearly parallel manner)\textsuperscript{38} to the inner tooth structures can be explained by the fracture-resistant properties of the enamel and dentin\textsuperscript{39, 40}.

What has been known so far, is that the internal enamel (the enamel near the DEJ where the rods extend within groups that are obliquely oriented to one another) shows strong resistance to fracture and that crack growth resistance increases from outside to inside (fracture toughness of outer enamel at the tooth’s surface 0.67 ± 0.12 MPa m\textsuperscript{0.5}, at inner enamel 2.62 ± 1.39 MPa m\textsuperscript{0.5}/mm)\textsuperscript{36, 38, 39, 41, 42}. The rise in crack growth resistance within the inner enamel is explained by several mechanisms of toughening, including crack bridging, crack deflection, and microcracking (i.e. the ability of the enamel microstructure to promote guided crack growth and arrest)\textsuperscript{34, 39}. Thus, it was expected to see that the MCs starting in the enamel would be stopped at the DEJ, which has unique biomechanical properties and provides a crack-arrest barrier for flaws formed in brittle enamel\textsuperscript{40, 43} by one of these fracture-resistant mechanisms.

Meanwhile, the dentin is structured with dentinal tubules surrounded by a thin mineral layer which develop

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**Figure 4.** An intricate star-shaped network of cracks after removing small isolated groups. **a1–a3** Projected density maps (bluish higher / brownish lower). **b1–b3** Distance to the nearest crack voxel along the line of sight (bluish further / brownish closer). **c1–c3** Three largest connected crack groups isolated and colour-coded (red – largest, green – 2nd largest, blue – 3rd).
Figure 5. Four healthy teeth in our study sample (a, b, c, d) shown from random side views. X-ray µCT data-cube projected density maps of convolutional neural network segmented enamel (green), dentin (blue), and cracks (red, intensity scaled as square root for enhancement) reveal an intricate inner structure.

Microscopic cracks under load\textsuperscript{40}. Incipient cracks in dentin can propagate into a “sea of microcracks” that triggers a series of strong extrinsic mechanisms of toughening (fracture toughness of dentin 1–2 MPa m\textsuperscript{0.5} in directions perpendicular and parallel to the tubules\textsuperscript{40,43}. The internal network of MCs revealed in our study justifies the term “sea of microcracks” mentioned above.

From the basics of mechanics, the stress concentration at the pre-existing crack depends on the crack shape and size as $\sigma_{\text{max}} = 2 \sigma \sqrt{l_c/l_{\rho}}$, where $2l_c$ is the length of pre-existing crack (assumed elliptical) and $l_{\rho}$ is its curvature at the tip. The threshold stress $\sigma_{\text{th}} \approx E/2\pi$ for crack propagation is when $\sigma_{\text{max}} = \sigma_{\text{th}}$; $E$ is the Young modulus. The observed dental crack planes – preferentially along and perpendicular to the compressive stress experienced by tooth – reduce propensity for crack propagation at smaller angles (along the direction of the tooth) as implied from experiments with building materials subjected to compressive stress\textsuperscript{44}.

It is important to emphasize that only healthy undamaged teeth (with or without visible MCs on the outer surface) were selected as study sample. All the examined premolars were removed atraumatically (i.e. the root of the tooth was gently separated from the periodontal ligament using a special instrument; low pressure and constant force; no lateral, rotational and traction movements) by an experienced oral surgeon\textsuperscript{45}. Although the teeth were not loaded (i.e. not subjected to external forces during the study), the network of MCs was still clearly visible as a continuous connective element in all examined samples. This could reflect the structural nature of the internal
network of MCs and lead to the hypothesis that it has a protective function (i.e. redistribution of forces).

The teeth in the mouth are subjected not only to masticatory forces, orthodontic stress during treatment with fixed appliances, but also to various parafunctions (e.g. bruxism), which can lead to occlusal overload and a higher risk of enamel damage\(^1\). There are two main groups of known tooth protection mechanisms that help to withstand lifelong stresses: a) accommodative function of the periodontium (soft and hard tissues that surround the root of the tooth and change their anatomical and physiological features in response to occlusal forces)\(^{46}\), and b) the structural and mechanical properties of the enamel (the most highly mineralized tissue of the human body consisting of 96% mineral, 1% protein, and 3% water by weight)\(^{34}\). Enamel has all the characteristics of an anisotropic material, i.e. its mechanical properties such as hardness (~3–6 GPa), elastic modulus (70–120 GPa), and brittleness vary depending on the location, chemical components, and arrangement patterns of the enamel rods\(^{1,36,47–49}\), and thus contribute to the redistribution of the forces (guiding crack arrest, microcracking phenomenon)\(^{34,39}\) and to the protection of the tooth’s internal structures against damage.

The results of this study show that cracks can still propagate through the DEJ into the dentin, so the DEJ could no longer be considered as an absolute barrier to MC arrest. However, despite the fact that occlusal stresses can reach and affect not only the enamel but also the dentin, the tooth retains its structural integrity. Therefore, based on the results of the study, we hypothesise that the continuous connective MC network found in the healthy tooth may be the missing link in answering the question of how the tooth is able to withstand the full range of occlusal forces.

**Figure 6.** Same as Fig. 5, but the top (occlusal) view of our four healthy teeth study sample. An intricate star-shaped network is visible.
Forces without damage and fragmentation – a third tooth protection mechanism for structural stability and stress adaptation.

There is evidence in the literature of correlation between the tissue dehydration and the dynamic dimensional changes within dentin and enamel\textsuperscript{50}, as well as between dehydration and the fatigue crack growth resistance\textsuperscript{51}. Due to the characteristics and working principle of the X-ray scanner used in this study\textsuperscript{34}, the samples could not be stored in an aqueous media during the scanning procedure. Although it is not known exactly what effect the storage of the samples in non-hydrated media may have had on the cracks located in the tooth crown, it has been already shown that the enamel MC width and length values were not affected by the dehydration that occurs during the preparation of the samples for SEM scanning and observation (no new MCs were also registered)\textsuperscript{1,52}.

In conclusion, the approach presented – tooth X-ray \(\mu\)CT in combination with CNN assisted segmentation – allows for a non-destructive and comprehensive 3D characterisation of different severity MCs that can be located in various tooth layers. Anatomical features of the tooth, such as enamel thickness, surface convexity or roughness, are no longer considered to be a barrier to MCs’ analysis with the described method. The revealed tendency for the MCs is to connect with each other and form a single star-shaped network (longitudinally in relation to the main axis of the tooth) covering most of the tooth, while single cracks appeared to be planar (interconnected manifolds) rather than narrow threads. Thus, disclosure of the MCs network inside a healthy tooth (with or without visible MCs on the buccal surface) indicates that the cracks could be considered as one of the structural and possibly functional (i.e. redistribution of forces) elements of the tooth, with a protective rather than a detrimental function.

Revelation of MCs as a new tooth structural element extends our understanding of the cracking pattern in natural hard materials and provides insights into how biologically inspired structures could be designed to predict the propagation of cracks in solids. From a clinical point of view, there is a need to revise the definition of MC that has been used so far, to re-evaluate the role and impact of these cracks on the integrity and longevity of the tooth, and to develop new algorithms for the monitoring and treatment of teeth with MCs in daily clinical practice.

Methods

Samples and study design

Human maxillary premolars extracted for orthodontic reasons were included in the study. The primary teeth selection criteria were as follows: a) intact (i.e. healthy, undamaged) buccal, palatal/lingual, and contact surface enamel with no white spots, signs of dental fluorosis or enamel hypoplasia; b) no pre-treatment with any chemical agents (such as hydrogen peroxide); c) no previous orthodontic, endodontic or restorative treatment; d) atraumatic tooth extraction procedure; e) specimens correctly stored after extraction. The secondary criterion for the teeth selection was the presence of visible and invisible enamel MCs on the buccal enamel surface\textsuperscript{1,34,53} (Fig. 7). The protocol of the study was approved by the Ethical Review Board of Vilnius University (Lithuania) and all the experiments were performed in accordance with relevant guidelines. The teeth were prepared in accordance with the guidelines of the International Organization for Standardization (ISO/TS 11405; 2003)\textsuperscript{54} and were used with the patients’ informed consent and permission to utilize the obtained data for research purposes.

Data acquisition

X-ray \(\mu\)CT scans with ZEISS Xradia 520 Versa X-ray microscope (Pleasanton, CA 94588, USA) were used in the current study of a 3D distribution of cracks and other features in the teeth specimens\textsuperscript{34}. A detailed description of the scanning procedure is presented in previously published study\textsuperscript{34}. The final result of the scanning procedure was four data-cubes of \(\sim 10^3\) mm\(^3\) (\(\sim 2000^3\)) voxels, containing values stored as 16-bit integers, with voxel edge of \(\sim 5\) \(\mu\)m.

Data preparation for analysis

For visualisation purposes, the data-cubes were resampled to a common voxel scale and aligned to principal contact surfaces of the teeth with \(\sim 10\) \(\mu\)m scale. In Fig. 8 axes \(x, y, z\) were aligned as indicated (\(x\) along palatal, \(y\) along contact, and \(z\) along vertical extent of tooth). For detailed mapping of MCs, the vertical slice of the tooth was divided into three horizontal slabs of equal height corresponding to 1/3 (cervical third), 2/3 (middle third) and 3/3 (occlusal third) of the tooth surface. Tooth surface division was based on dental examination methodologies and different enamel quality and mechanical properties of the individual thirds\textsuperscript{1,4,7,36,53,55,56} (Fig. 8).

As each tooth has four surfaces of different convexity (Fig. 7), enamel thickness, distance to dentin and pulp, four sectors (buccal, contact right, palatal, contact left) were identified for analysis\textsuperscript{57,58} (Fig. 8 (b–h)). Transitions from convex (buccal, palatal) to flatter tooth surfaces (contact right, contact left) were selected as reference points to define sectors. The dashed magenta lines connected the upper reference point of the right contact surface to the lower reference point of the left contact surface and vice versa. This resulted in 3 \(\times\) 4 3D slab sectors used for
examination of MCs of each tooth. In each of the slab sector in Fig. 8, the following structures could be identified:

- Enamel (visible as light-grey shaded area), dentin (dark-grey area surrounded by enamel), pulp (black area in the central part of the tooth), cracks (in the enamel, dentin, or both layers), scanning artefacts (large black cylinder, \( \mu \text{CT playback “rays” on top of the tooth} \)), enamel discolouration at the bottom.

Although the principal components of the tooth can be identified by the grey level of voxel values with an eye, a straightforward selection of voxels to isolate tooth components would result in rather poor quality, e.g. cracks, pulp, and outside of the tooth have same numerical values. Therefore, we trained a CNN to identify voxels (pixels in each slice since we processed data-cube as slices for this purpose) which belong to these categories: 1) cracks, 2) enamel, 3) dentin, 4) air.

A single tooth segmentation results (slices of the tooth along \( z \) axis) of previous study\(^{34} \) as hand crafted and eye verified labels were used to train a new CNN model\(^{39} \) for segmentation of the other three teeth in the sample. The CNN model was implemented using “TensorFlow”\(^{60} \) and achieved \( \sim 99.5\% \) accuracy on both training and validation images without over-fitting. An example of network’s predictions is presented in Fig. 9.

Each slice image (\( \sim 1000 \times 1000 \) pixels) was cropped into \( 512 \times 512 \) pixel size overlapping tiles as suitable for CNN’s input, which yielded same size pixel classification maps that were assembled back to the size of the slice. The segmentation of each tooth data-cube was performed three times, taking slices perpendicular to \( x \), \( y \), and \( z \) axis. A voxel was identified as crack if it was classified as crack in at least two planes. The remaining voxels were classified as enamel, dentin, or air. A slice-by-slice fill-in was performed to restore enamel and dentin areas (as if tooth had no cracks) using “scikit-image”\(^{61} \) \texttt{restoration.inpaint} method on dilated mask of cracks.

Data-cube manipulations were performed using “Numpy”\(^{62} \) while visualisations were created with “Matplotlib”\(^{63} \) libraries. “SAOImageDS9” tool\(^{64} \) was used for visual assessment of iterative model training and teeth segmentation task. Although some minor segmentation artifacts can be noticed, the results of the study are robust. The connected crack groups were isolated and filtered by size using “cc3d” library\(^{65} \).
Figure 8. X-ray µCT data-cube cuts of a tooth without visible enamel microcracks on the buccal surface. Enamel is light-grey, dentin – dark-grey, and tooth outside, pulp and cracks – black. a Vertical slice in $x, z$ plane at $y$ position, as indicated by horizontal dotted yellow line in panels (b – h). a Horizontal dashed red and dotted cyan lines show positions $z = 1 – 4$ of slices in $x, y$ planes, displayed as images in panels (b – h). a Horizontal slabs selected between dashed red lines are indicated as 1/3, 2/3, 3/3. In panels (b – h) dashed magenta lines cut-out four identified sectors (buccal, contact right, palatal, contact left). Field of view is $\sim 8 \times 8 \text{ mm}^2$. One pixel corresponds to $\sim 10\,\mu\text{m}$. 
Figure 9. Example of X-ray µCT data-cube slice-by-slice segmentation with convolutional neural network (CNN) to produce data-cubes of structural elements of tooth. a CNN’s predictions on slice image with enamel (yellow), dentin (brown), cracks (dark blue), and air (black) indicated. b Binary mask of cracks, which was used to fill-in segmentation image shown in (c), resulting in isolated areas of enamel and dentin presented in (d) as if there were no cracks in the tooth. One pixel corresponds to ∼10 µm.

References


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Author contributions statement
I.D. and M.M. conceived the experiment, D.N. and A.V. conducted the experiment and performed the measurements, I.D., D.N., S.J. and M.M. analysed the results, I.D. and D.N. wrote the manuscript. All authors reviewed the manuscript.

Competing interests statement
The authors declare no competing interests.
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